

CLAIMS

1. A method of quantifying a first nucleic acid in a first biological source, comprising the steps of:

- 5 (a) combining said first biological source containing said first nucleic acid with a known amount of a second biological source containing a second nucleic acid;
- 10 (b) extracting from said combination said first nucleic acid and said second nucleic acid to form a combined nucleic acid extract;
- 15 (c) adding to said combined nucleic acid extract a first detectable probe which is specific for said first nucleic acid and a second detectable probe which is specific for said second nucleic acid;
- 20 (d) amplifying said combined nucleic acid extract by PCR means with a first set of primers which is specific for said first nucleic acid and a second set of primers which is specific for said second nucleic acid;
- (e) quantifying at various PCR cycles during said amplification a detectable signal released independently from said first detectable probe and said second detectable probe;
- 25 (f) extrapolating the results of step (e) to calculate the amount of said first nucleic acid in said first

biological source and the amount of said second nucleic acid in said second biological source; and

- 5 (g) evaluating accuracy of said calculated amount of said first nucleic acid determined in step (f) by comparing said calculated amount of said second nucleic acid in step (f) with said known amount of said second nucleic acid used in step (a).

- 10 2. The method according to claim 1 further comprising the step of adjusting said calculated amount of said first nucleic acid determined in step (f) of claim 1 by a factor determined by comparing said calculated amount of said second nucleic acid in step (f) of claim 1 with said known amount of said second nucleic acid used in step (a) of claim 1.

- 15 3. The method according to claim 1, wherein said first biological source is selected from cell-associated virus, including virus particles, subparticles, or free nucleic acid, and cell-free virus, including serum, plasma, or other media containing virus particles, 20 subparticles, or free nucleic acid.

4. The method according to claim 1, wherein said first nucleic acid is selected from viral DNA or RNA from cell-associated or cell-free virus.

5. The method according to claim 1, wherein said second biological source is selected from cell-associated virus, including virus particles, subparticles, or free nucleic acid, and cell-free virus, including serum, plasma, or other media containing virus particles, subparticles, or free nucleic acid.

6. The method according to claim 1, wherein said amplification is conducted by PCR or RT-PCR.

7. The method according to claim 1, wherein said amplification is conducted using two sets of primers, wherein a first set of said primers is specific for said first nucleic acid and a second set of said primers is specific for said second nucleic acid.

8. The method according to claim 1, for quantifying nucleic acid in HCV, comprising the steps of:

- (a) combining said HCV containing said first nucleic acid with a known amount of BVDV containing a second nucleic acid;
- (b) extracting from said combination said first nucleic acid and said second nucleic acid to form a combined nucleic acid extract;
- (c) adding to said combined nucleic acid extract with a first detectable probe which is specific for said first nucleic acid and a second detectable probe which is specific for said second nucleic acid;

(d) amplifying said combined nucleic acid extract by PCR or RT-PCR means;

5 (e) quantifying at various PCR cycles during said amplification a detectable signal released independently from said first detectable probe and said second detectable probe;

10 (f) extrapolating the results of step (e) to calculate the amount of said first nucleic acid in said HCV and the amount of said second nucleic acid in BVDV; and

15 (g) evaluating accuracy of said calculated amount of said first nucleic acid determined in step (f) by comparing said calculated amount of said second nucleic acid in step (f) with said known amount of said second nucleic acid used in step (a).

9. The method according to claim 8 further comprising the step of adjusting said calculated amount of said first nucleic acid determined in step (f) of claim 1 by a factor determined by comparing said calculated amount of said second nucleic acid in step (f) of claim 1 with said known amount of said second nucleic acid used in step (a) of claim 1.

25 10. A method of determining the effect of a compound on the replication of a first nucleic acid of a first biological source, comprising the steps of:

(a) combining said compound with a known amount of cell culture system to produce a first combination, wherein

said first nucleic acid of said first biological source is capable of replication;

(b) after a time period combining said first combination with a second biological source containing a
5 second nucleic acid to produce a second combination;

(c) extracting from said second combination said first nucleic acid and said second nucleic acid to form a combined nucleic acid extract;

(d) adding to said combined nucleic acid extract with
10 a first detectable probe which is specific for said first nucleic acid and a second detectable probe which is specific for said second nucleic acid;

(e) amplifying said combined nucleic acid extract by PCR or RT-PCR means;

15 (f) quantifying at various PCR cycles during said amplification a detectable signal independently released from said first detectable probe and said second detectable probe;

(g) extrapolating the results of step (f) to
20 calculate the amount of said first nucleic acid and said second nucleic acid in said second combination;

(h) evaluating accuracy of said calculated amount of said first nucleic acid determined in step (f) by comparing said calculated amount of said second nucleic acid in step
25 (f) with said known amount of said second nucleic acid used in step (a);

(i) determining the effect of said compound on the replication of said first nucleic acid by comparing said amount of said first nucleic acid as determined in step (g) with the amount of said first nucleic acid
5 determined separately in the absence of said compound.

11. The method according to claim 10, wherein said first biological source is selected from cell-associated hepatitis C virus, including virus particles, subparticles, or free nucleic acid, and cell-free hepatitis
10 C virus, including serum, plasma, or other media containing virus particles, subparticles, or free nucleic acid

12. The method according to claim 10, wherein said compound is capable of inhibiting or interfering with Hepatitis C virus life cycle.

15 13. The method according to claim 10, wherein said second biological source is selected from cell-associated virus, including virus particles, subparticles, or free nucleic acid, and another cell-free virus, including serum, plasma, or other media containing virus
20 particles, subparticles, or free nucleic acid.

14. The method according to claim 10, wherein said extraction means is selected from any suitable DNA or RNA extraction technique, including matrix-based single-well spin or vacuum column, or multiple-well extraction
25 plate, or solution-based extraction methods

15. The method according to claim 10, wherein said first virus is HCV and said second virus is BVDV.

16. A method of simultaneously screening a plurality of compounds for their effect on the replication of a whole or part of a genome of a first biological source, comprising the steps of:

5 (a) placing in one or a plurality of wells said whole or part of a genome of said first biological and a medium suitable for replication of said genome;

 (b) adding to each said well one or more of said compounds;

10 (c) adding to each said well a known amount of a second biological source as an internal control;

 (d) using extraction means to extract together from each said well a first nucleic acid and a second nucleic acid to produce a combined nucleic acid extract from each
15 well;

 (e) amplifying and quantifying during the amplification process said first nucleic acid and said second nucleic acid in each well;

 (f) determining the effect of each of said compounds
20 on the replication of said whole or part of a genome of a first biological source using the results from step (e).